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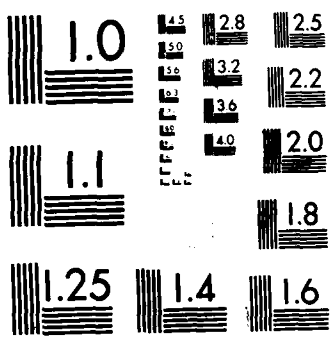

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FISSION TRACK ASSAY FOR FISSILE NUCLIDES IN TISSUE

Final Comprehensive Summary Report for Phases I and II of the Fission Track Assay for Fissile Nuclides in Tissue

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Technical Report

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Prepared for
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Final Comprehensive Summary Report for Phases I and II of the Fission Track Assay for Fissile

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Fundamental techniques for a Fission Track Assay for Fissile Nuclides in Tissue have been developed under Contract No. DNA ~~001-82-C-0100~~. All of the scientific goals have been reached within the time allotted by the funded project. Accomplishments can be summarized as follows:

a. Pu and U have been chemically separated from each other and from bulk contaminants frequently encountered in ashed tissue specimens.

The preparation of sample-detector assemblies has been standardized and conditions for neutron irradiation, subsequent track etching and quantification have been established.

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19. ABSTRACT (Continued)

c) Program controlled procedures have been developed to eliminate erroneous interpretations of fission track images and to guarantee the statistical validity of the track quantitation.

d. The background due to external contamination with uranium has been reduced to acceptable levels.

e. A survey of Pu-distributions in livers, kidneys, spleens and lungs and skeletons of experimental animals has been performed.

f. Reproducibility and accuracy of the assay have been tested on small beef liver samples tagged with 1.0 and 0.1 fCi ^{239}Pu and on dry, ^{239}Pu-containing liver powder obtained from the National Bureau of Standards.

g. The Pu distribution and average concentration of six livers of humans from Southern Utah and Northern Utah have been determined and the results were compared with those obtained from the same organs by α-spectrometric methods.

Presently, the lower limit of the ^{239}Pu quantitation using a reported thermal neutron fluence of 5×10^{17} n/cm^2 is approximately 1 fg or less than 10^{-16} Ci. Thus, Pu resulting from 1/10,000 of the maximum permissible whole body burden of 4×10^{-8} Ci could be quantitated in a 100 mg bone specimen, assuming a uniform skeletal deposit of ^{239}Pu.

.000004

CONVERSION TABLES

Conversion factors for U.S. Customary to metric (Si) units of measurement.

Multiply ---------→ To get ←---------	by ---------→ by ←---------	To get Divide
barn	1.000,000 x E - 28	$meter^2 (m^2)$
Curie	3.700,000 x E + 1	*giga becquerel (GBq)
micron	1.000,000 x E - 6	meter (m)

*The becquerel (Bq) is the SI unit of radioactivity; 1 Bq = 1 event/sec.

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SECTION 1

INTRODUCTION

During the first phase of development of the "Fission Track Assay for Fissile Nuclides in Tissue", fundamental techniques had to be established that could later be used to quantitate the presence of fissile nuclides in small samples of biological tissue such as surgical and autopsy tissue blocks or biopsy specimens. During Phase II results obtained during the first phase had to be applied to actual small tissue samples and the distribution of Pu in certain target organs had to be established.

Since irradiation with thermal neutrons produces a high fission yield from ^{239}Pu, ^{233}U and ^{235}U, these two elements had to be separated prior to neutron bombardment. The main emphasis was directed toward quantitation of ^{239}Pu. However, the possibility of determining ^{233}U and ^{235}U introduced into the specimen with natural uranium or from manmade sources was required as well.

Some groundwork for developing highly sensitive fission track assays for ^{239}Pu was done by Larsen and Oldham (La75), who later abandoned that project because of interference by natural uranium, a ubiquitous contaminant even in the higher grade reagents used. With the present project, work on that subject was resumed, and the basis for a workable fission track assay of ^{239}Pu and $^{235/233}U$ in tissue was provided.

SECTION 2

PHASE I: DEVELOPMENT OF THE FISSION TRACK ASSAY

GOAL

The goal was to develop a quantitative assay for fissile Pu that could be applied to the analysis of soft and hard tissue blocks. The desired lower limit of detection was less than 1 fCi. At the end of this Phase, the feasibility of the proposed assay had to be demonstrated and a protocol for routine analysis had to be available.

ACHIEVEMENT

In spite of many technical difficulties arising primarily from contamination of the applied reagents with uranium, that task has been successfully completed. Progress of theoretical and practical work on Phase I has been documented in a summary report (Sept. 1984).

In addition, work performed during Phase I as sponsored by DNA resulted in two publications in the open literature (Sm84, Wi83).

AREAS OF RESEARCH

The basic technique for determining femtogram quantities of fissile nuclides by fission track methods seems straightforward. It consists of the chemical separation of the respective fissile nuclides in massless form (i.e., no measurable self-absorption), their deposition on a suitable substrate and preparation of a substrate-detector assembly. The assembly is then irradiated with a known fluence of thermal neutrons, followed by chemical etching of the resulting tracks on the detector and their quantitation. In reality, this procedure is very difficult to execute.

Our efforts to provide a workable method proceeded simultaneously in four different, but mutually dependent, areas of research:

1. a chemical study during which the chemical separation and quantitative recovery of the fissile nuclides in essentially massless form was developed;
2. a technical study concerned with minimizing the background due to the ubiquitous presence of uranium in the reagents used and in the environment;

3. a technical study of the preparation of source-detector assemblies; and

4. a physical and engineering study of all aspects of fission track detection and quantitation.

ISOLATION OF NUCLIDES

Development of the isolation procedure was carried out initially under normal laboratory conditions, applying techniques of professional cleanliness. Nanogram quantities of the nuclides Pu and U which could be quantitated conveniently by scintillation counting were used in these early experiments. Two methods were available for the separation of Pu and U from other mineral contaminants, liquid-liquid extraction and ion exchange chromatography. Of these two, ion exchange chromatography is clearly superior for the quantitative separation and recovery of femtogram quantities of the nuclides from bulk contaminants and from each other. The method is based on the formation of strong, negatively charged chlorocomplexes of Pu(IV) and U(VI) at high HCl concentrations. Both elements are adsorbed on a strongly basic anion exchange resin while other mineral contaminants pass through. After washing, the Pu chlorocomplex is destroyed with HBr of density $\rho = 1.5$ and the Pu is eluted, leaving U on the column. Uranium is then eluted with 0.1 N HCl. The column effluent is reduced in volume to a few microliters and readied for transfer to a suitable substrate. A flow chart of the separation is shown in Figure 1.

Optimum conditions with respect to resin type, column size, acid concentration, washing time, flow rate and valence stabilization were determined experimentally. The interference caused by the presence of high phosphate concentrations--as introduced with skeletal tissue--was studied. Formation of neutral or positively charged Pu-phosphate complexes which are not adsorbed by the resin was avoided by increasing the HCl concentration during the sorption process. The increase in $[H^+]$ converts phosphate ions into undissociated, and therefore nonreactive, phosphoric acid. Leakage of ^{239}Pu or ^{233}U during the sorption phase was 2% or less. At the level of 1×10^{-9} Ci of applied activity, the ^{239}Pu or ^{233}U recovery was > 95%. The recovery at lower levels of activity was explored using ^{237}Pu, whose specific activity is approximately 2×10^5 times greater than that of ^{239}Pu. However, full use of the higher specific activity could not be made because of contamination of this preparation with ^{239}Pu. The recovery of Pu decreased successively when the number of Pu atoms applied was less than 1×10^{10}, as demonstrated by the data in

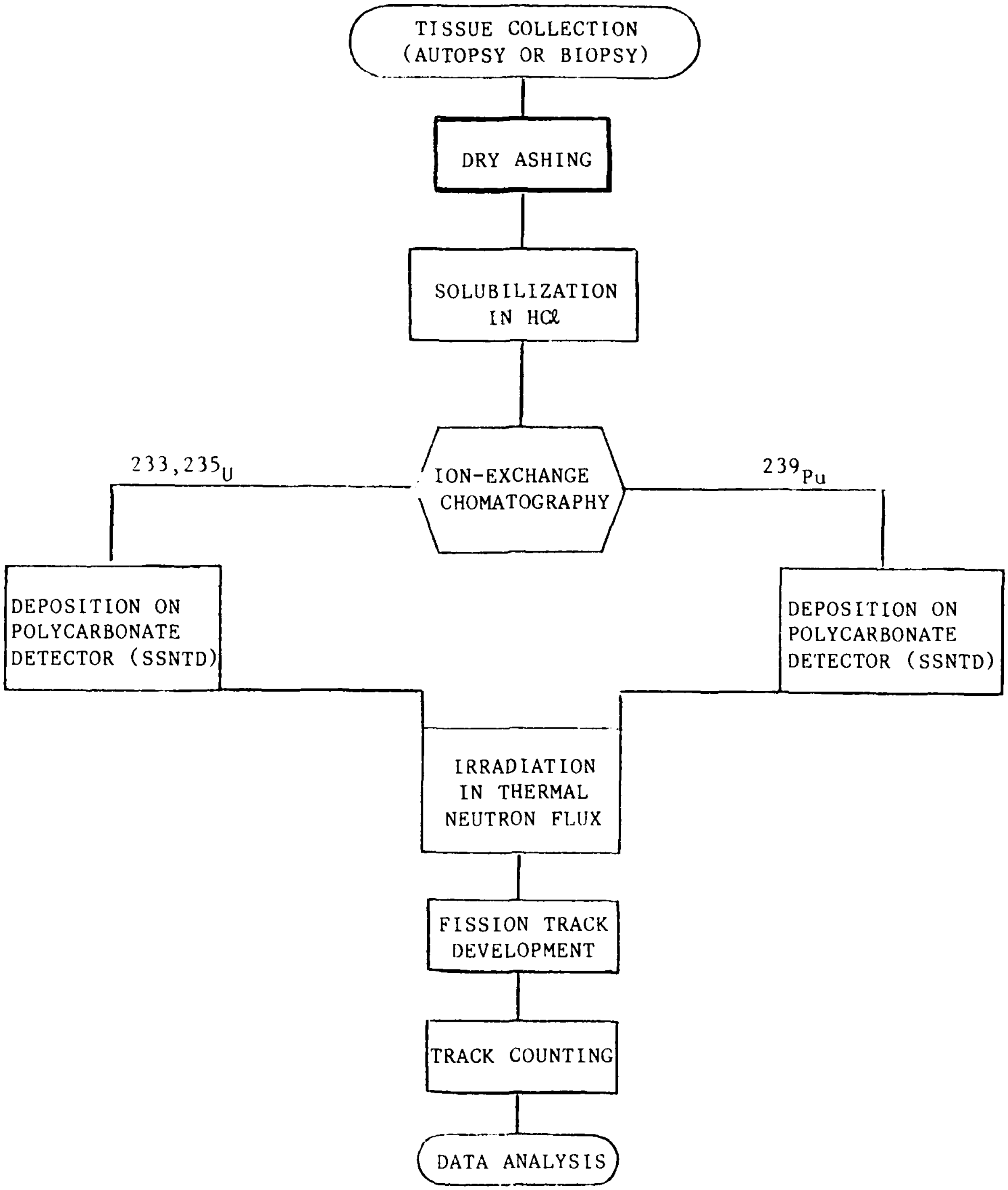

Figure 1. Flow diagram for the preparation of samples, the chromatographic separation of Pu and U from bulk contaminants, the fission track development and data analysis.

Table 1. A search for a suitable isotopic tracer to monitor the recovery of ^{239}Pu at levels below the limit of detection through conventional chemical methods was unsuccessful. However, Zr(IV) showed a sorption-desorption behavior identical to that of Pu(IV) under the conditions applied. Therefore, ^{95}Zr has been used until now as a conditional tracer for Pu recovery. The ^{95}Zr obtained by the laboratory had been separated from fission products and tended to increase the background in the fission track analysis. A source of ^{88}Zr prepared from ^{88}Sr has been found, but no purchases have yet been made because of the high cost. Figure 2 shows the elution spectra of Pu and U and corresponding elution of the conditional tracer.

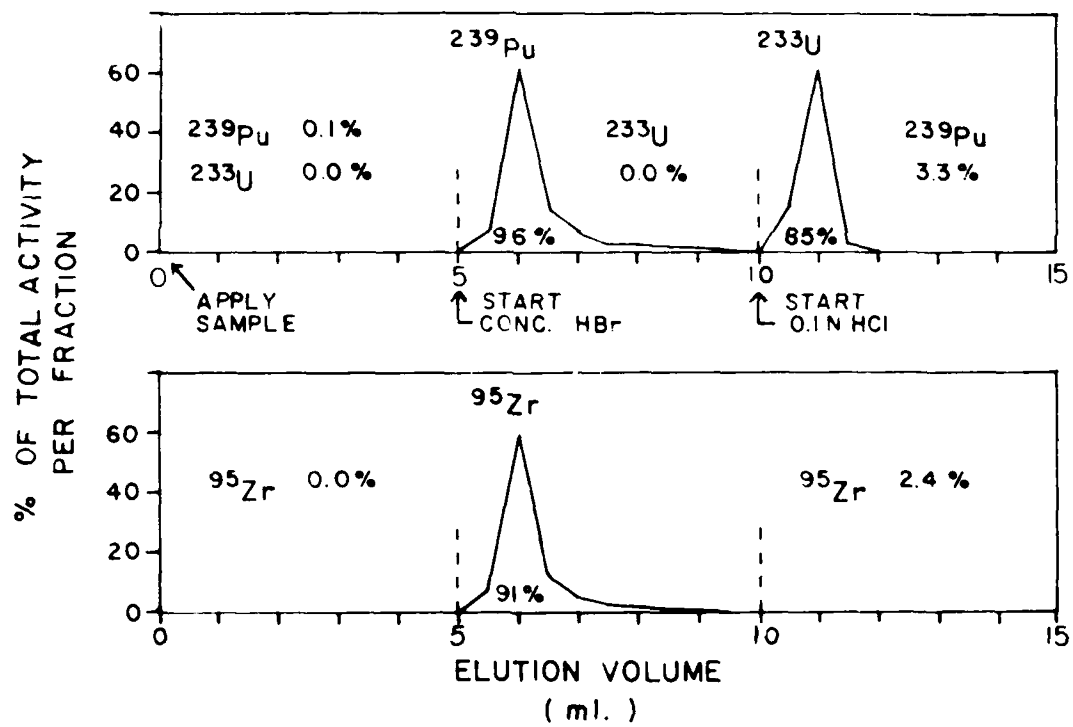

Figure 2. Elution spectra of Pu and U obtained under present experimental conditions of anion exchange chromatography. For comparison, the elution spectrum of ^{95}Zr, which has been proposed as a conditional tracer for Pu, also is shown.

Table 1. Recovery of Pu from resin. Influence of number of Pu atoms.

APPLIED	RECOVERY	
	Uncapped Resin	Capped Resin
4×10^{11} atoms	95%	89%
4×10^{10} atoms	87%	88%
4×10^{9} atoms	83%	88%
4×10^{8} atoms	64%	93%

The possible mechanisms leading to losses at very small quantities of ^{239}Pu were investigated by extrapolating theoretically the data obtained experimentally at the level of approximately 1×10^{-9} g of Pu to the level of 1×10^{-15} g or lower. Mechanisms of the following reactions were studied.

A) Disproportionation reactions among Pu species.

Since only Pu(IV) forms a strong negatively-charged chlorocomplex that is adsorbed and retained by the resin, the three principal reactions among the various species of uncomplexed Pu and their corresponding rate equations have been studied:

1. $Pu(III) + Pu(V) \rightleftarrows 2\ Pu(IV)$
2. $Pu(III) + Pu(VI) \rightleftarrows Pu(IV) + Pu(V)$
3. $Pu(IV) + Pu(VI) \rightleftarrows 2\ Pu(V)$

An assessment of the time scale for this type of reaction can be obtained by considering pure Pu(IV) at t = 0.

It follows that the rate of disappearance of Pu(IV) is

$$\frac{d[IV]}{dt} = 2\left\{-K_{-1}[IV]^2 + K_1[III][V]\right\} - K_{-2}[IV][V] + K_2[III][VI] + K_{-3}[V]^2 - K_3[IV][VI] \quad (1)$$

In this equation, the Roman numerals stand for the Pu concentrations present in those valence states, and K_i, K_{-i} (i = 1 to 3) are the respective rate constants for the forward and backward reactions. These rate constants have the following values.

$$K_1 = 4.4 \times 10^{-2}\,[H^+] \qquad K_{-1} = 3.0 \times 10^{-5}\,[H^+]^{-3}$$

$$K_2 = 2.7 \qquad K_{-2} = 3.4$$

$$K_3 = 1.2. \times 10^{-7}\,[H^+]^{-3} \qquad K_{-3} = 2.3 \times 10^{-3}\,[H^+]$$

For $[H^+]$ = 10 M, this gives:

$$K_1 = 0.44 \qquad K_{-1} = 3.0 \times 10^{-8}$$

$$K_2 = 2.7 \qquad K_{-2} = 3.4$$

$$K_3 = 1.2 \times 10^{-2} \qquad K_{-3} = 2.3 \times 10^{-2}$$

The time to reach the vicinity of the inflection point, where the decomposition of Pu(IV) is greatly accelerated, is obtained by approximating the first part of the complex reaction above with the second order reaction as follows:

$$2\ \mathrm{Pu(IV)} \rightarrow \mathrm{Pu(III)} + \mathrm{Pu(V)}$$

$$\frac{d[IV]}{dt} = -2k_{-1}[IV]^2 \tag{2}$$

which yields

$$t = \frac{[IV]_o - [IV]}{[IV][IV]_o} \cdot \frac{1}{2k_{-1}} \tag{3}$$

The conclusion of this investigation was that, due to the second order nature of the reactions among the Pu species, the half-life of Pu(IV) is proportional to the inverse of the concentration. For trace amounts on the order of 10^{-15} M (as expected in our experiments), the half-life is very long compared with the few hours duration of the experiment. Thus an appropriate holding oxidant added to the sample before application to the resin will provide the required initial conditions for essentially complete oxidation to Pu(IV). Two oxidants were considered, NaCℓO and $C\ell_2$ (gas), both of which provided $\frac{Pu[III]}{Pu[IV]}$ ratios of the order of 10^{-4} to 10^{-5}

at the concentration that was used. Of these two, Cl_2 (gas) was chosen because of the lower probability of uranium contamination. A more detailed treatment of A) was included as an appendix to our progress report for the period of 1 April 1983 to 30 June 1983 (Sm83).

B) Conditions leading to possible losses of Pu or U during the anion exchange separation.

1. Resin capacity and adsorption isotherms.

Trace amounts of ionic species are considered to bind to synthetic ion exchangers with linear binding isotherms, assuming binding to the average site in the resin. This linearity implies that the distribution coefficient for the anionic species A^- (such as the chlorocomplexes of Pu or U) remains constant regardless of the tracer load. However, our experiments, summarized in Table 1, have shown decreasing recoveries as the Pu load decreased below the level of 1×10^{10} atoms. The quoted assumption of linear binding isotherms has been made only for resin loads that are 3 to 5 orders of magnitude larger than applied in the present case. We assumed that there are some adsorption sites whose affinity for A^- is higher than average and that their number is insignificantly small under normal loading conditions, but becomes significant for the trace levels anticipated in our experiments. Since nothing is known about the nature of these postulated sites, we have to make the following assumptions:

a. The high affinity site has the same chemical properties as the average adsorption site, but the local concentration of adsorptive groups is much higher.

b. The relative order of affinities toward the individual average adsorption site for different ions is unchanged at the high affinity site.

Thus by pretreating the exchanger with an ionic species, i, known to have a distribution coefficient D_i larger than D_{Pu} or D_U under elution conditions, one should cover all of these sites. With all of the high affinity sites blocked, linear isotherms should be observed at extremely low resin loads.

Accordingly, prior to sample loading, high affinity sites were capped with Cd^{++}, excess Cd (bound to the average site) was eluted, and the previous recoveries of Pu were restored, as indicated by results presented in Table 1.

2. Influence of phosphates on adsorption of Pu and U.

During loading of actual samples at strongly elevated HCl concentrations (12.5 M), phosphates are primarily present as H_3PO_4 or $H_2PO_4^-$ with the more basic phosphates present at insignificant levels. At the level of $C\ell_2$ present in the sample, one can assume that all of the Pu is present in the Pu(IV) valence state. Even under these assumptions, the number of different Pu complexes theoretically can be quite large. In view of the relatively large column capacity, the only mechanism for Pu-leakage should be the formation of uncharged or positively charged complexes. In principal, the fractions of such species can be calculated for a given set of conditions. Unfortunately, a satisfactory numerical analysis requires knowledge of the stability constants of complexes with higher coordination numbers and these are largely unknown. However, it can be shown that the fraction of positive and neutral Pu-species is independent of the initial concentration of Pu. Accordingly, experimental results obtained at 10^{-13} M concentration are also predicted at concentrations of 10^{-18} M. Since experimental data indicated that the Pu retention at high HCℓ concentration was good, it is implied that the negatively charged species predominate. Thus results obtained at "high" Pu concentrations will also hold at much lower Pu values, provided the HCℓ/phosphate ratio remains high.

Again, more detailed information was given in our progress report for the period of 1 April 1983 to 30 June 1983 (Sm83).

REDUCTION OF BACKGROUND

The buildup of natural uranium, which contains about 0.7% fissile ^{235}U, will cause serious interference with the Pu-fission track analysis. Unlike α-spectrometry, etched fission tracks provide no information on the parent nuclide. Uranium is ubiquitously distributed through the environment, and probably poses the most serious threat to the success of the project. For

actual sample analysis, the source of contamination can be divided into two groups:

a. Introduction of uranium with chemical reagents or by dissolution from laboratory ware, especially glass. This type of U-accumulation was reduced by using only very small quantities of reagent of highest purity and by using either quartz or special plastic laboratory ware. However, a number of chemical reagents were not available at the desired level of purity. In some cases, even the highest level of purity (ultra-pure) did not meet the extremely rigid requirements with respect to uranium contamination, especially some of the acids. In these cases, reagents were either synthesized under clean-room conditions or otherwise prepared from purified gaseous feedstocks that had a sufficiently low level of U-contamination. For example, hydrobromic acid was catalytically prepared from H_2 and Br_2, Cl_2-gas was used as a holding oxidant, and N_2O_4 gas together with O_2 was applied in the oxidation of residual organic contaminants.

   The quantity of resin used for the ion exchange separation was reduced from 250 mg dry resin to 125 mg; therefore, the amount of reagents could be reduced to about one half the original quantity. A secondary water purification system that uses source water of 15-17 megaohm resistivity prefiltered through a filter of 1 μm pore size has been added. The source water is passed again through activated charcoal, an additional ion-exchange cartridge and a final filter of 0.2 μm pore size. The final resistivity of this water is between 19.5 and 20.5 megaohm.

b. All developmental work, especially the work performed with ^{239}Pu or ^{233}U at levels of activity between 10^{-9} and 10^{-11} Ci had been done under conditions of normal laboratory practice. As work shifted to much lower levels of activity, established procedures were carried out in a clean-room. Contamination from airborne particles in the clean-room is minimized by maintaining positive pressure of air filtered through a HEPA-system (0.5 μm pore size, 99.5% efficiency) using a special, limited access room. Personnel working in this area are required to wear a complete set of special, dustfree clothing including shoe-covers, cap and face mask and non-powdered gloves. The clean-room is equipped with a fume hood used for chemical procedures and other work that requires sample manipulation. The fume hood also houses a small furnace with a quartz-lined chamber for

ashing of actual tissue samples. Final preparation steps of source-detector assemblies, such as volume reduction of column effluents and plating and drying of samples, are carried out in a secondary enclosure within the clean-room that--with respect to the room--is also under positive pressure of air filtered through a secondary HEPA-filter of 0.2 μm pore size. Incomplete data on the overall efficiency of the clean-room indicate that conditions for maintaining sufficiently low levels of background are adequate. However, this is a potential problem area which will require constant vigilance.

PREPARATION OF SOURCE-DETECTOR ASSEMBLIES

After column chromatography, Pu is contained in approximately 5 ml of concentrated HBr and U in the same volume of 0.1 N HCℓ. Deposition of the nuclides on suitable substrates requires evaporation of the acid and wet ashing of possible residues in strongly oxidizing media to remove traces of organic material. Oxidation previously had been carried out with a mixture of HNO_3 and $HC\ell O_4$. This has now been replaced by low temperature ashing in a stream of filtered O_2 and N_2O_4 gas. Insoluble impurities would destroy the uniform distribution of fissile nuclides (and consequently of the fission tracks) on the detector, which would make counting of fission tracks difficult. In addition, these impurities cause flaking which interferes with the quantitative recovery.

Initially, two methods of deposition onto a support base were considered: electrodeposition on Pt-discs and manual transfer to polycarbonate or quartz slides. Electrodeposition from a dimethylsulfoxide-HCℓ medium onto polished Pt-discs was successful, but even the highest purity Pt available produced too high a U background, and neutron activation of the Pt through the ^{194}Pt (n,γ), ^{195m}Pt and other reactions required long cooling times. The preferred method is to transfer the dissolved nuclide directly onto polycarbonate or quartz supports (slides). Uniform dispersion over a well-defined area can be achieved by slow evaporation of the droplets on support slides coated with an albumin, gelatin, or other liquid adsorbing layer. A developed fission track image of a section of such a droplet is shown in Figure 3.

FISSION TRACK DETECTION AND QUANTITATION

Irradiation of source-detector assemblies with thermal neutrons is carried out at the MIT Research Reactor. The reactor yields a high quality (high cadmium ratio) thermal neutron flux of 8 x 10^{12} n/cm$^2\cdot$sec. The thermal

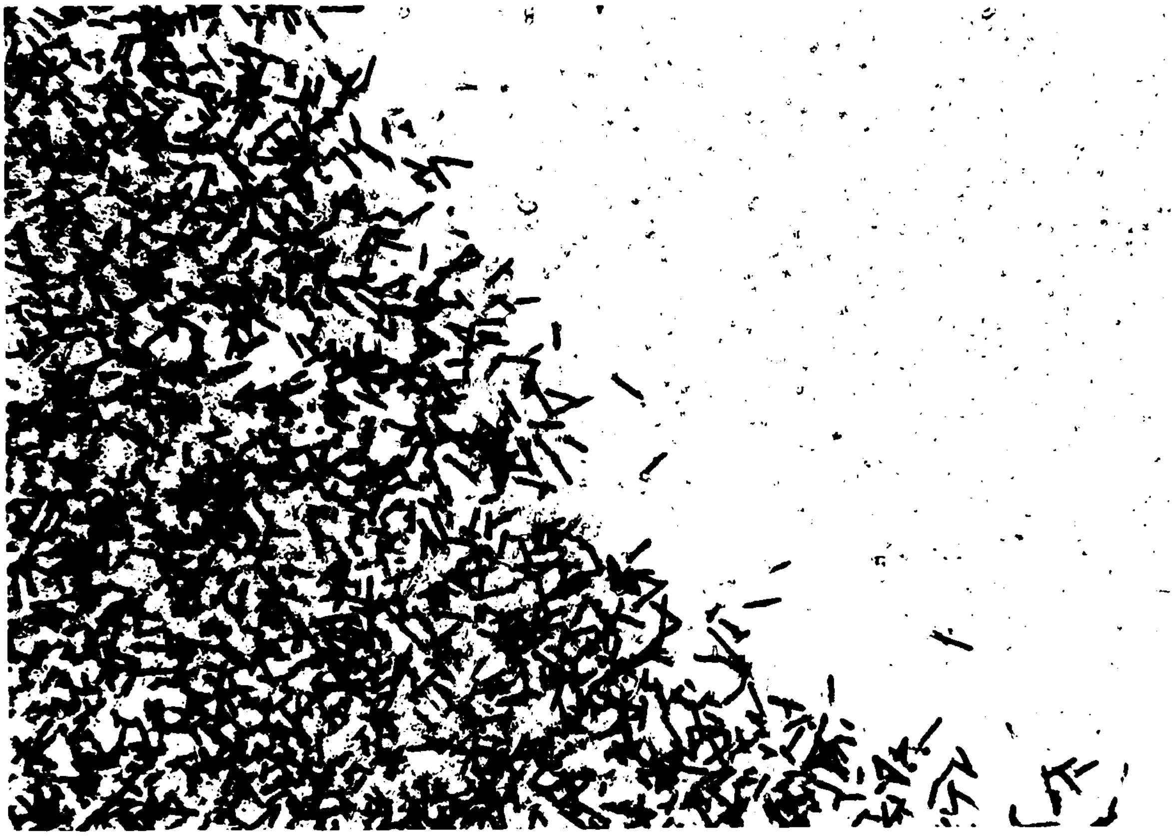

Figure 3. The figure shows part of the perimeter of the fission track image of a droplet of Pu as obtained after column chromatography.

neutrons then induce fission in the fissile nuclides $^{233,235}U$ and ^{239}Pu in the sample. Etching of the latent fission tracks for appropriate times with 6.25 N KOH at 70°C produces visible fission tracks which can be quantitated. The etching also produces fission tracks derived from contamination with natural uranium and shallow pitmarks induced from (n, α) and neutron knock-on

reactions. If low concentrations of fissile nuclides in the sample require high neutron fluences, the increasing number of fission tracks from natural uranium and the surface pitmarks from (n, α) and neutron knock-on produce a background that could strongly interfere with the quantitation of ^{239}Pu originated fissions in the sample. Also, impingement of neutrons damages the detector surface such that etching removes a certain thickness, and as a result, the track detection efficiency is diminished. This removal is proportional to the fluence, and its magnitude is expressed by the Bulk-Etch Rate in units of μm/min versus fluence. Figure 4 shows the experimentally determined Bulk Etch Rate for the range of 10^{15} to 10^{18} n/cm^2. Since the Bulk Etch Rate increases with increasing fluence, the etching time is decreased from 12 minutes at 1×10^{16} n/cm^2 to 6 minutes at 5×10^{17} n/cm^2. Interference due to damage of the surface by α-impingement was minimized by contrast enhancement of the fission tracks with a dye. Simultaneously, the influence of the shallow surface damage was reduced considerably by wetting and filling the pitmarks with an agent having a refractive index close to that of the polycarbonate detector during viewing. The effect of this procedure on fission track images generated at increasing fluences is shown in Figure 5.

Most experiments were limited to neutron fluences of up to 10^{17} n/cm^2 but fluences of 5×10^{17} n/cm^2 which require 17.5 hours of irradiation time are possible. Higher fluences can be justified only in exceptional cases because of cost and background interference.

Theoretical Aspects and Practical Studies of Track Detection

The following subjects were studied regarding track detection and quantitation.

1. Review of theoretical aspects of fission track detection.

   Since, due to the conservation of momentum two fragments per fission are emitted in opposite directions, one fragment per fission produced from a source in which all of the fissile atoms are at the interface between the source and the detector will produce a detectable track. Such ideal conditions are approximated in electrodeposited sources of low activity. However, for reasons given earlier, electrodeposition from actual column effluents was not possible. Actual deposits of fissile atoms are

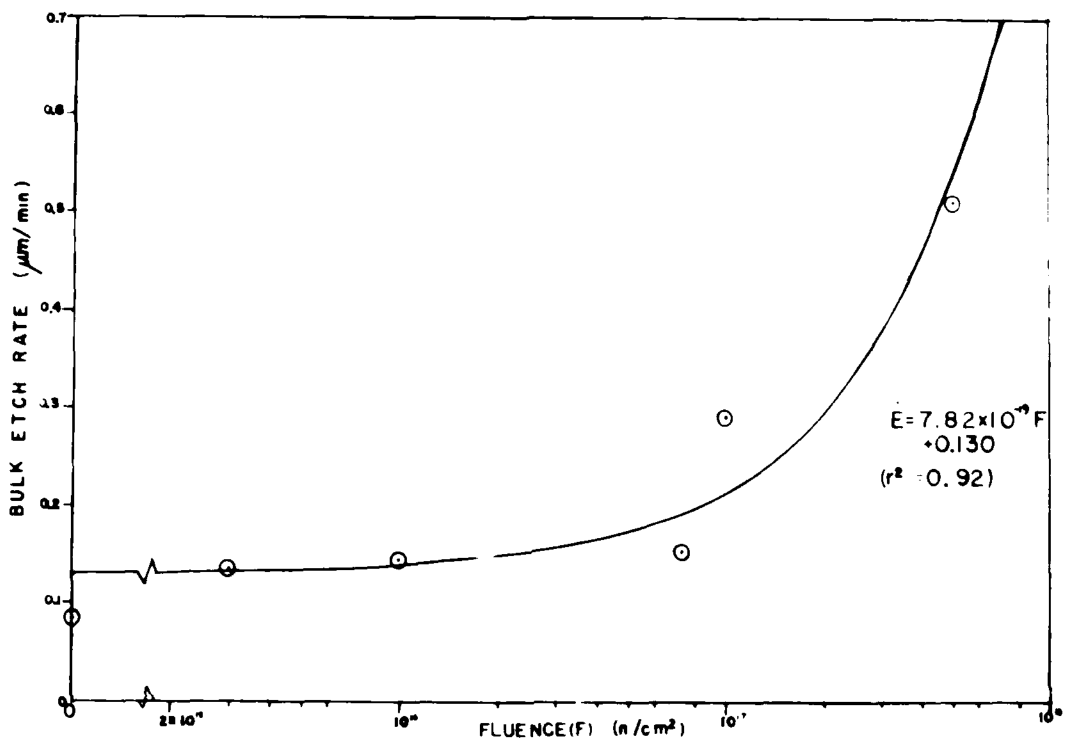

Figure 4. Dependence of the Bulk Etch Rate on neutron fluence. Bulk Etch Rate is a measure of detector matrix destroyed during neutron irradiation and removed from the detector surface during the etching procedure. The Etch Rate is expressed in μm removed/min of etching time.

approximately uniformly distributed throughout the source matrix, so the probability p(x) that a fission fragment of range R originating at a distance x below the surface will cross the plane interface between the source matrix and the detector can be expressed by

$$p(\theta,x) = \frac{2\,\Omega(\theta,x)}{4\pi} \tag{4}$$

where $\Omega\,(\theta,x)$ is the solid angle associated with the fission fragments emitted from a point at a distance x from the interface, which enter the detector at angles equal to or less than θ, as illustrated by Figure 6.

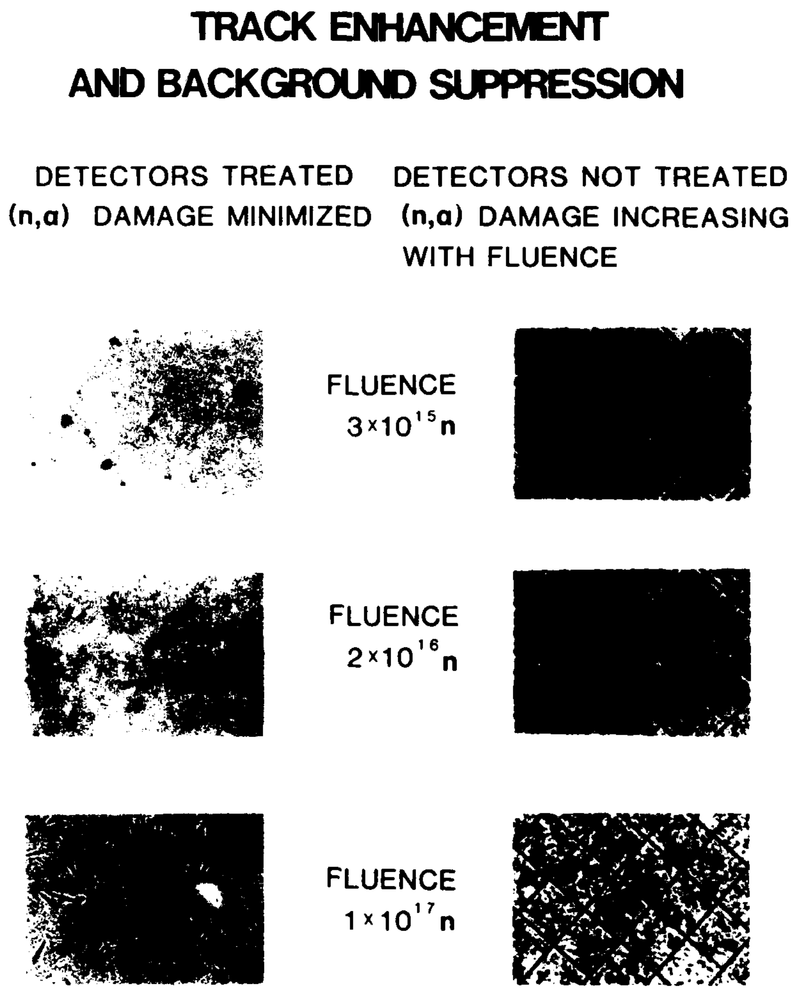

Figure 5. The figure shows the patterns of increasing background features as the neutron fluence is elevated. The reduction of the background and contrast enhancement of the etched fission tracks are clearly visible.

Assuming isotropic emission of fission fragments the angle θ_o corresponds to the maximum solid angle Ω (θ_o, x) that geometrically limits the number of fission fragments entering the detector.

Thus, integrating p (θ, x) from 0 to θ_o,

$$p(x) = \frac{\int_o^{\theta_o} \int_o^{2\pi} R^2 \sin\theta \; d\theta \; d\phi}{2 \pi R^2}$$

$$= 1 - \frac{x}{R} \tag{5}$$

where, $\cos \theta_o = \frac{x}{R}$ as shown in Figure 6.

The number of theoretically possible tracks N_T registered per square micron in a detector with 100% efficiency can then be expressed as

$$N_T = N_f \int_o^R p(x)dx$$

$$= \frac{N_f R}{2} \tag{6}$$

where N_f is the number of fissions induced per cubic micron originating from any point $x < R$.

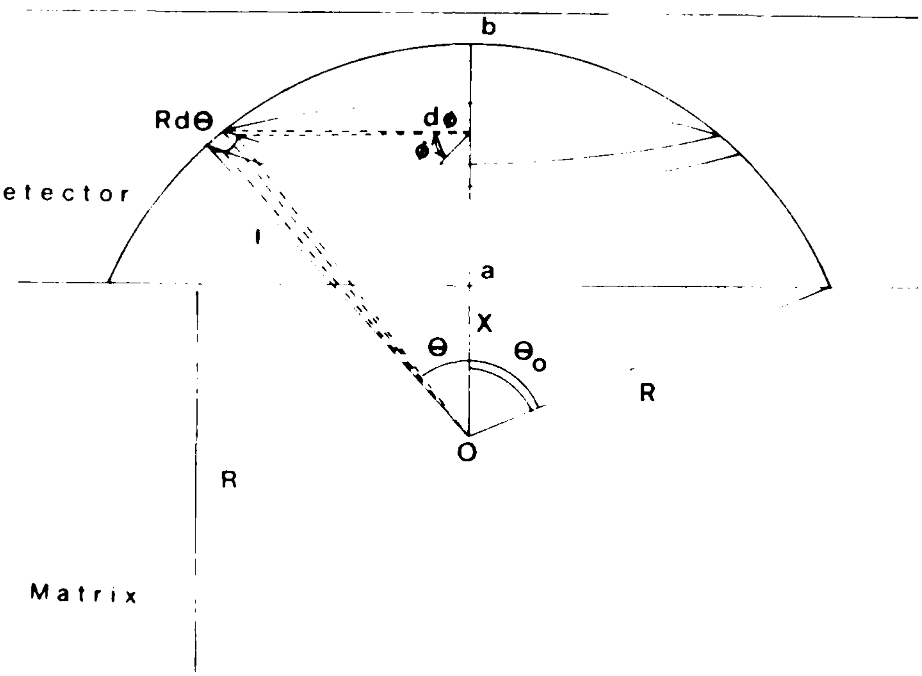

Figure 6. Geometric model used to determine the probability that a track of range R originating at a distance X ≤ R below the detector surface will reach the detector.

The observed track density TD is related to the theoretical N_T by the equation

$$TD = \varepsilon \ N_T \tag{7}$$

where ε is the detection efficiency.

Substituting N_T given by equation (6) into equation (7), the latter becomes

$$TD = \varepsilon \ \frac{N_f R}{2}. \tag{8}$$

The quantity N_f is related to the number of fissile atoms per unit volume n by the expression

$$N_f = n \ \sigma_f \Phi \tag{9}$$

where σ_f is the fission cross-section of the fissile nuclide for thermal neutrons, and Φ is the thermal neutron fluence which is given by

$$\Phi = \int_o^t \zeta(t)dt \tag{10}$$

where $\zeta(t)$ is the time dependent thermal neutron flux at a fixed position in the reactor.

Substituting equation (9) into equation (8), the observed track density can be conveniently expressed as

$$TD = \frac{\varepsilon\sigma_f \Phi R}{2} n = kn \tag{11}$$

where $k = \frac{\varepsilon\sigma_f \Phi R}{2}$.

Thus, the observed track density is directly proportional to the number of fissile atoms per unit volume of the sample matrix. The proportionality factor k which appears in equation (8) can easily be determined by using known quantities of the nuclide in source matrices prepared under the same conditions as the actual sample and irradiated with the same neutron fluence.

2. Human visual track quantitation.

For low track densities, quantitation can be performed by visually counting the tracks per unit area using a microscope. A sufficiently large number of fields have to be counted to obtain statistically valid

data to compensate for nonuniform track density distribution, and the total area must be known. Similarly, fields of known area can be photographed, and the number of tracks and average track density evaluated from the prints made from these photographs.

3. Instrumental detection.

Human visual track counting is labor intensive. The laboratory is equipped with a Quantimet (QTM) 720 Image Analyzer (Cambridge-Imanco, Monsey, N.Y.), which can be used for automatic detection and counting of etched fission tracks. The system includes an automatic scanning stage and is linked to a PDP 11/03 minicomputer (Digital Equipment Corp.) by way of high speed interfaces, permitting feature analysis and a statistical analysis of the data. Details regarding the automatic fission track counting techniques have been described in our quarterly report for the period of 1 October 1982 - 31 December 1982 (Sm83).

4. Linearity of response using the QTM-720.

It is important to select neutron fluences that produce a range of track densities which increases proportionally with the concentration of the nuclide in the source matrix. The range of linearity was determined by two methods:

a. From detectors exposed to a mixture of fission fragments and α particles using various exposure times and a source of electrodeposited ^{252}Cf. No neutron irradiation was used.

b. From detectors prepared by n irradiation of a series of 12 plastic wafers containing ^{239}Pu concentrations ranging from 1×10^{14} to 5×10^{11} atoms of ^{239}Pu/g of substrate at neutron fluences that ranged from 5×10^{15} to 1×10^{17} n/cm^2. Figure 7 shows the result of one of these experiments, corresponding to a fluence of 5×10^{15} n/cm^2. This demonstrates a linear response of the QTM-720 at average track densities up to 3×10^3 tracks/mm^2. A more convenient track density for the QTM was in the range from 100 to 700 tracks/mm^2.

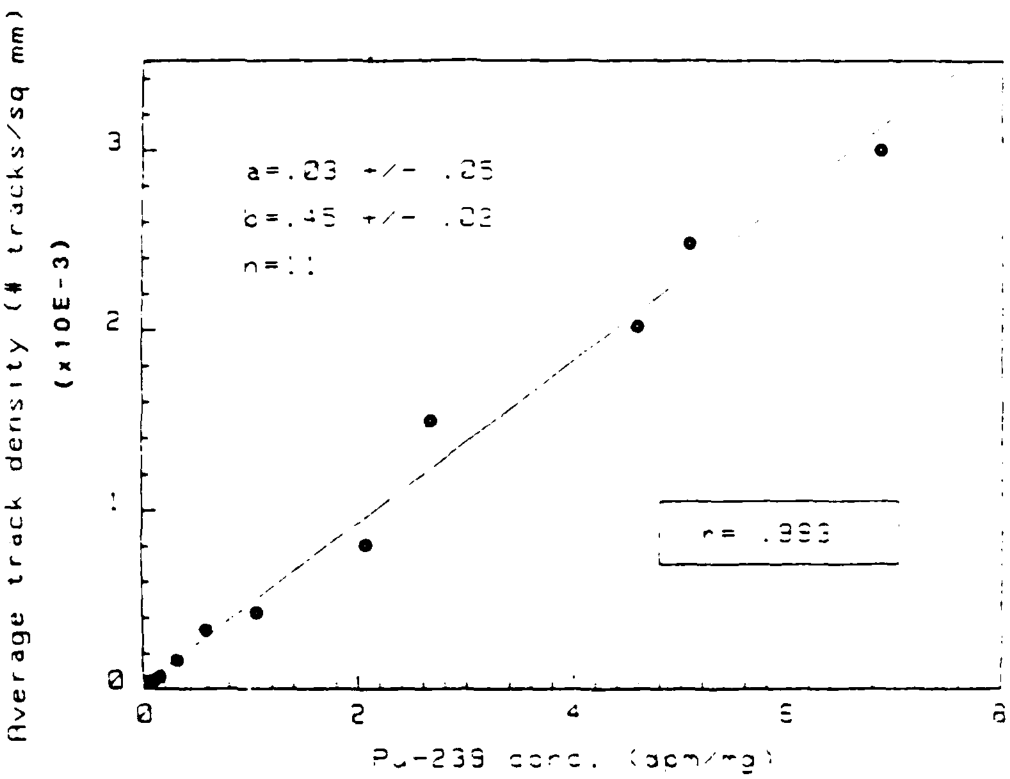

Figure 7. The average track density (tracks/mm^2) as determined by instrumental track counting with the QTM is plotted against the Pu concentration of the source matrix. Linearity of track detection efficiency exists over a concentration range of at least one order of magnitude. Data obtained at a fluence of 5×10^{15} n/cm^2.

SECTION 3

PHASE II: COMPARISON OF FISSION TRACK COUNTING TECHNIQUES WITH STANDARD RADIOCHEMICAL ASSAY (1 YEAR)

GOAL

The laboratory began work on Phase II of the Fission Track Project on 1 July 1984. The goal of Phase II is: a) to adapt the methods and procedures devised in Phase I to the analysis of actual tissue specimens; b) to address the potential problem of heterogeneity of the Pu-distribution in those organs that are considered main targets for future analysis ie., liver, lung, bone and possibly kidney and spleen.

ACHIEVEMENT. Present Status

Our experimental efforts during this phase have been directed toward three areas: a) the transition from single experiments to routine techniques with well established but rigidly controlled protocols; b) an extensive survey of the distribution of Pu and U in those organs for which future analysis of surgical or biopsy tissue blocks is anticipated; and c) the processing of actual tissue specimens that are likely to contain Pu and/or U embedded in an inert tissue matrix which is neither well defined nor uniform.

a. Adaptation to routine procedures requires that the sample can be processed and analyzed for Pu and U with a reasonable degree of reproducibility and accuracy through rigid quality control and dust-free working conditions. These requirements were easily met with respect to chemical separation, preparation or procurement of ultra-clean reagents and working conditions which avoid contamination of reagents and specimen with environmental uranium. Initial difficulties in maintaining uniformity in shape, thickness and homogeneity of the source-substrate assemblies that must be irradiated have been overcome. The fraction of unreadable fission track images is now less than 2% of the processed sources. Thus, the method presently applied is suitable for routine operations.

b. Within the body, Pu and U are transported via the bloodstream and distributed in certain target organs in well established proportions. It is accepted that the final deposition after a secondary distribution is independent of the initial mode of entry into the body, be it by inhalation, puncture wound or intestinal absorption or by direct introduction

into the blood stream by injection. Only the rates at which deposition in the target organ occurs are different, varying with the route of exposure and the chemical form and inert matrix of the contaminant. However, the distribution within the final target organ may not be uniform, although it is our experience that the uniformity in the distribution increases as the level of radioactivity decreases. To investigate the uniformity of the final distribution of Pu within the target organs projected for future fission track analysis, liver, kidney, spleen, lung and bone specimens obtained from experiments with canines were used. Small random samples were taken as follows: 5 or 6 specimens from each liver, 4 from each kidney pair, spleen and lung. These tissues were analyzed radiochemically; the results were expressed as the concentration of the nuclide found in each random sample divided by the average concentration of all samples taken from the same animal. Since the distribution may vary with the age at time of exposure and with the time after exposure, the survey was performed on animals with a human age equivalent of 18 to 23 years at time of exposure and of 45 years or older. Also, the time after exposure was varied from times less than 1 year to more than 10 years. A summary of these data is presented in Table 2. Included in the table are the number of organs analyzed, the age in months of the experimental animals at the time of exposure (for which the human age equivalents are given above), the time from exposure to death and the range of concentration ratios of individual organ samples (C_i) to the mean concentration in the respective organ ($\bar{C}$). A fractional standard deviation for all $C_i/\bar{C}$ values of each group was calculated.

With this survey, a sufficiently large number of specimens can be used to estimate statistically the expected degree of variation within individual organs. The weight of individual tissue specimens analyzed ranged from about 1g to 2.9g, somewhat larger than samples expected from surgical or autopsy tissue blocks for future fission track analysis. However, variations associated with a future scaling down to one fifth are not expected to be vastly different. This view is corroborated by the results shown later in this report.

Table 2. Relative Concentration of Pu in Various Organs. (Concentration in individual samples/average concentration in whole organ).

Organ	Number of Organs Analyzed	Average Age at Exposure (months)	Average Time from Exposure to Death (months)	Range of Ci/$\bar{C}$ †		Fractional Standard Deviation for all Ci/$\bar{C}$ Data
Liver	6	18*	4 ± 4.6 (7-363 d)	0.88	1.26	0.13
	9	18	114 ± 22	0.42	1.38	0.22
	15	60**	46 ± 9.2	0.76	1.38	0.10
Kidney	6	18	4 ± 4.6 (7-363 d)	0.68	1.21	0.10
	9	18	134 ± 22	0.28	1.52	0.29
	15	60	46 ± 9.2	0.34	1.22	0.11
Spleen	6	18	4 ± 4.6 (7-363 d)	0.92	1.07	0.04
	10	18	134 ± 20	0.39	2.2	0.28
	13	60	48 ± 17	0.72	1.45	0.13
Lung	6	18	4 ± 4.6 (7-363 d)	0.68	2.5	0.35
	9	18	129 ± 18	0.38	1.86	0.30
	15	60	46 ± 9.2	0.68	1.19	0.14

* Average age of an 18 month old beagle is equivalent to a human age of 18-23 years

** Average age of a sixty month old beagle is equivalent to a human age of 45 years or more.

A similar survey was performed with 30 Pu-contaminated skeletons. Each skeletal member was analyzed and the relative concentration of Pu in each bone (i.e., fractional activity of Pu in each bone divided by its respective fractional skeletal mass) was calculated. The relative concentration within the skeleton varied by a factor of about four, but the difference among the same bones was less than 0.2. Bones most likely to be considered for tissue block fission track analysis, such as lumbar vertebrae, humeri, femora, ulnae and pelves, were further sectioned into 3

(for the lumbar) to 8 (for the humerus and femur) sections and analyzed radiochemically. Again, the relative concentrations within each set of sections differed by a factor of 4.5, but the difference among corresponding sections was less than 2 in bones with a high biological turnover rate and about 0.3 for biologically less active bones such as the ulna. For the iliac crest (pelvis), proposed in the original contract as the most likely candidate for surgical biopsy, the difference was 0.35. Thus, as long as specific bone sections are analyzed by the fission track method, presently estimated variations due to local differences in the Pu(U) concentration are well within acceptable limits.

This initial survey indicates that distributional differences in the nuclide concentrations are probably not an impediment to future application of the fission track assay. However, a confirmation of these estimates by the new and much more sensitive fission track method is essential and is provided below.

c. Appropriate steps have been taken to conduct an actual comparison of fission track determined concentrations of Pu in small tissue sections with those determined by analysis of large tissue specimens.

Three series of experiments have been conducted to test the reproducibility and accuracy of the assay with actual tissue samples. In the first series, 12 pieces of beef liver (each with about 1g of tissue) were taken. Four of these were tagged with 0.1 fCi and four with 1 fCi of ^{239}Pu. The specimens were dried, ashed and analyzed by the fission track method. Fission tracks obtained with the untagged liver sections were subtracted as background from the tagged specimen. The recovery for 0.1 fCi sections was 86.2% ± 2.8%; that for the 1 fCi samples was 88.9% ± 2.0% with an overall recovery of 87.5% ± 2.6%. In addition, six samples of Pu-tagged dry liver powder having a nominal activity of 0.03 pCi $^{239}Pu/g$ were analyzed, two by the conventional α-spectrometric method and four by the fission track assay. The average activity determined by α-spectrometry was 0.032 pCi/g. Using the NBS value of 0.03 pCi/g as the actual concentration, the recovery by the fission track method was 87.2% ± 3.3%. These results show that accurate Pu-determinations can be made with the fission track method, provided the level of exogenous uranium contamination is controlled by the use of ultrapure reagents and a clean environment.

Since an earlier attempt to analyze small aliquots of HNO_3-ashed large tissue digests that had been prepared under normal laboratory conditions had failed because of the high content of uranium (see Progress Report for the period of 1 July - 31 Sept. 1984), random samples of gram quantities of tissue have been taken before wet ashing from tissues received by our environmental laboratory. The original store consisted of 212 such specimens, with 18 bone sections, 93 liver, 51 lung, 46 kidney, and 4 heart specimens for this analysis. A number of these tissues have been analyzed by the fission track assay and compared with the data obtained by α-spectrometry on large quantities of tissue.

The Pu-content of six livers (25 samples total) was determined. The respective results are shown in Tables 3 and 4. Variations in Pu concentration of individual samples were no larger than those observed in the preparations shown in Table 2. A comparison with the Pu data obtained on the same tissue by α-spectrometric analysis of the whole tissue (several hundred grams/organ) showed good agreement between the two methods.

An abstract of a scientific paper on this subject was submitted to DNA and cleared for presentation at the International Conference on Nuclear Analytical Techology in Karlsruhe, Germany (Br85). The presentation was given to a large audience. From ensuing discussions it can be concluded that there is great interest in application and further development of this method.

Table 3. Fission track analysis of human liver specimens (Southern Utah - Fluence 1 x 10^{17} neutrons/cm^2).

		Sample Weight (g)	Gross Tracks	Net Tracks (sample)	fCi Pu (sample)	fCi Pu (g)	$\bar{X}$	$^{239+240}Pu$ (fCi/g) *
83 S5	a	1.24	1064	744	0.632	0.51		
	b	1.01	942	622	0.528	0.52		
	c	1.03	817	497	0.422	0.41		
	d	0.82	736	416	0.353	0.43	0.46	0.47
83 S6	a	1.32	1816	1496	1.27	0.98		
	b	0.80	1333	1013	0.86	1.07		
	c	1.02	1439	1119	0.95	0.93		
	d	0.88	1427	1107	0.83	0.94	0.98	1.20
83 S7	a	0.91	779	459	0.39	0.43		
	b	1.27	862	542	0.46	0.36		
	c	1.07	885	565	0.48	0.45		
	d	0.89	744	424	0.36	0.41		
	e	0.94	838	518	0.44	0.47	0.42	0.38
Std 1 fCi	a		1164					
(3100 fission)	b		1191	= 38% efficiency				
	c		1179					
			1178					

BKG Determination

1 BKG each with 2 sample columns except for last (83 S7 c,d,e) which had 3 sample columns. BKG = 5 ml HBr run through column and treated like actual sample.

83 S5	(a+b)	294
	(c+d)	331
83 S6	(a+b)	327
	(c+d)	302
83 S7	(a+b)	318
	(c+d+e)	346
		$\bar{X}$= 320

*$^{239+240}Pu$ in whole organ as determined by α-spectrometry.

Table 4. Fission track analysis of human liver specimens (Northern Utah - Fluence 5×10^{17} neutrons/cm^2).

		Sample Weight (g)	Gross Tracks	Net Tracks (sample)	fCi Pu (sample)	fCi Pu (g)	$\bar{X}$	$^{239+240}Pu$ (fCi/g) *
83 N25	a	1.36	7319	5845	1.07	0.79		
	b	0.94	6127	4653	0.86	0.91		
	c	1.20	6500	5026	0.92	0.77	0.82	0.85
83 N22	a	1.17	3192	1718	0.32	0.27		
	b	1.21	2054	1580	0.29	0.24		
	c	0.91	3009	1535	0.28	0.31		
	d	0.95	2663	1189	0.22	0.23		
	e	1.06	3146	1672	0.31	0.39	0.27	0.25
83 N23	a	0.84	5312	3838	0.76	0.84		
	b	0.92	6529	5055	0.93	1.01		
	c	0.97	6376	4802	0.88	0.91		
	d	1.13	7252	5778	1.06	0.94	0.92	0.96
Std 1 fCi	a		5502					
	b		5496	5440 average = 35.1% efficiency				
	c		5321					

BKG Determinations

One BKG each with the following groups of columns.

83 N22	(a+b)	1486
	(c+d+e)	1496
83 N23	(a+b)	1464
	(c+d)	1456
83 N25	(a+b+c)	1469
		$\bar{X}$ = 1470

*$^{239+240}Pu$ as determined by α-spectrometry.

SECTION 4

CONCLUSIONS

The basic problems associated with the development of the Fission Track Assay project have been solved. A workable procedure has been developed and the feasibility of the method has been demonstrated. However, constant vigilance with respect to maintaining acceptable background levels is in order. Future work will show if the method can be applied to the routine analysis of actual tissue specimens.

Based on the success of the previous two years, the principal investigator and his coworkers feel confident that the practical applications of the newly developed assay as proposed under the mission-oriented Phase III will be equally successful, and will help its funding agency fulfill its obligation to find solutions to nuclear fallout and exposure problems for both the military and civilian sectors.

SECTION 5

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